

**Translation**

**PATENT COOPERATION TREATY**

**PCT**

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**8 JUN 2006**

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**  
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>BIF023274/GP</b>		<b>FOR FURTHER ACTION</b>	See Form PCT/IPEA/416
International application No. <b>PCT/FR2004/000744</b>	International filing date (day/month/year) <b>25.03.2004</b>	Priority date (day/month/year) <b>28.03.2003</b>	
International Patent Classification (IPC) or national classification and IPC			
Applicant <b>INSTITUT PASTEUR</b>			

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

a. ☐ (sent to the applicant and to the International Bureau) a total of \_\_\_\_\_ sheets, as follows:

☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) \_\_\_\_\_, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

<input checked="" type="checkbox"/>	Box No. I	Basis of the report
<input type="checkbox"/>	Box No. II	Priority
<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/>	Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/>	Box No. VI	Certain documents cited
<input type="checkbox"/>	Box No. VII	Certain defects in the international application
<input type="checkbox"/>	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language \_\_\_\_\_, which is the language of a translation furnished for the purposes of:
- ☐ international search (Rule 12.3 and 23.1(b))
- ☐ publication of the international application (Rule 12.4)
- ☐ international preliminary examination (Rule 55.2 and/or 55.3)
2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages 1-30 \_\_\_\_\_ as originally filed/furnished
- pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- ☒ the claims:
- nos. 1-26 \_\_\_\_\_ as originally filed/furnished
- nos.\* \_\_\_\_\_ as amended (together with any statement) under Article 19
- nos.\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- nos.\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- ☒ the drawings:
- sheets 1/7-7/7 \_\_\_\_\_ as originally filed/furnished
- sheets\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- sheets\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_
- ☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages \_\_\_\_\_
- ☐ the claims, nos. \_\_\_\_\_
- ☐ the drawings, sheets/figs \_\_\_\_\_
- ☐ the sequence listing (*specify*): \_\_\_\_\_
- ☐ any table(s) related to sequence listing (*specify*): \_\_\_\_\_
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages \_\_\_\_\_
- ☐ the claims, nos. \_\_\_\_\_
- ☐ the drawings, sheets/figs \_\_\_\_\_
- ☐ the sequence listing (*specify*): \_\_\_\_\_
- ☐ any table(s) related to sequence listing (*specify*): \_\_\_\_\_

\* If item 4 applies, some or all of those sheets may be marked "superseded."

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement		
1. Statement			
Novelty (N)	Claims	15-20, 26	YES
	Claims	13, 24	NO
Inventive step (IS)	Claims	15-20, 26	YES
	Claims	1-14, 21-25	NO
Industrial applicability (IA)	Claims	1-26	YES
	Claims		NO
2. Citations and explanations (Rule 70.7)			
Reference is made to the following documents:			
D1: WO 02/083892 A (MARLIERE PHILIPPE; POCHET SYLVIE (FR); BOUZON MADELEINE (FR); CENT) 24 October 2002 (2002-10-24);			
D2: WO 03/025163 A (KAMINSKI PIERRE-ALEXANDRE; MARLIERE PHILIPPE (FR); COTAYA RACHEL) 27 March 2003 (2003-03-27);			
D3: DATABASE BIOSIS [online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2003, CHANG H K ET AL: "Directed evolution of Comamonas testosteroni GZ39 m-hydroxybenzoate hydroxylase for the synthesis of 4-substituted catechols." XP002297541 Database accession no. PREV200300546363;			
D4: WAN LIANGLU ET AL: "In vitro evolution of horse heart myoglobin to increase peroxidase activity" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 95, no. 22, 27 October 1998 (1998-10-27),			

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pages 12825-12831, XP002297538 ISSN: 0027-8424.

D1 describes a method for evolving a protein X in such a way as to modify the properties thereof. More specifically, D1 makes reference to kinases belonging to classes EC 2.7.1, nucleotidyl transferases belonging to classes EC 2.7.7 and, in particular, polymerases and phosphorylases belonging to classes EC 2.4.2 (D1, claim 2).

The technical problem that document D2 is intended to solve is that of producing nucleoside conversion enzymes and derivatives thereof that have broader enzymatic activity so as to enable diversification of the industrial production of these compounds (D2, page 2).

In order to provide recombinant enzymes capable of treating the widest variety of deviant substrates, recombinant N-deoxyribosyltransferases (EC 2.4.2.6) from various strains of *Lactobacillus* were isolated and cloned. This variety of enzyme has broader specificity and is produced via mutations in the wild-type genes or using chimeras of said wild-type genes (D2, page 3).

Moreover, it is already known that nucleoside analogues constitute a family of molecules that are active in the treatment of many bacterial, viral, parasitic and fungal infections as well as in anti-tumour chemotherapy (D2, page 1).

In view of documents D3 and D4, the use of directed *in vitro* evolution to enhance the properties of an enzyme or

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

modify its specificity for a substrate are widely known in the prior art.

1. The present application does not fulfil the requirements set forth in PCT Article 33(1) because the subject matter of claims 13 and 14 does not comply with the requirement of novelty defined in PCT Article 33(2).

In light of the observations set out above, the subject matter of said claims cannot be differentiated from the disclosures in D1.

2. The present application does not fulfil the requirements set forth in PCT Article 33(1) because the subject matter of claims 1-12, 14, 21-23 and 25 does not involve an inventive step as defined in PCT Article 33(3).

The only difference between D1, which is considered to be the closest prior art, and claim 1 of the present international application is that, in D1, random mutagenesis is carried out *in vivo* in auxotrophic cells (D1, step (b)), while, in the present application, the DNA is mutated before being converted into auxotrophic cells.

The problem that the present invention is intended to solve can therefore be considered to be that of enhancing the prior art by providing an additional protein evolution method, in particular, for N-deoxyribosyltransferase (DTP).

Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
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- 2.1 The solution proposed in claim 1 of the present application is not considered to be inventive (PCT Article 33(3)), for the following reasons:

In light of D2, the production of a DTP that has broadened enzymatic activity was an urgent requirement in view of the medical importance of nucleoside analogues.

It follows that, in light of D1, it would have been obvious for a person skilled in the art, aware of documents D3 and D4, to carry out the method in claim 1.

- 2.2 The same argument applies *mutatis mutandis* to the subject matter of corresponding independent claims 14 and 21, which are consequently not inventive either.

- 2.3 Dependent claims 2-12 do not contain any features which, in combination with the features of any one of the claims to which they refer, might define subject matter that fulfils the PCT requirement of inventive step (see documents D1 and D2 and the corresponding passages cited in the search report).

3. The subject matter of claim 26 is novel and the combination of features in claims 15-20 is not found in the prior art and cannot be derived in an obvious manner therefrom.

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Box No. V

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As a result, these claims fulfil the requirements  
of PCT Article 33(2) and 33(3).